

Methods of Treating Dry Eye Disorders

This application claims priority to U.S. Provisional Application, U.S.
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The present invention is directed to the treatment of dry eye disorders.
In particular, the present invention is directed to the use of certain cytokine
synthesis inhibitors in the treatment of dry eye and other disorders requiring
10 the wetting of the eye in mammals.

Background of the Invention

Dry eye, also known generically as *keratoconjunctivitis sicca*, is a
15 common ophthalmological disorder affecting millions of Americans each year.
The condition is particularly widespread among post-menopausal women due
to hormonal changes following the cessation of fertility. Dry eye may afflict an
individual with varying severity. In mild cases, a patient may experience
burning, a feeling of dryness, and persistent irritation such as is often caused
20 by small bodies lodging between the eye lid and the eye surface. In severe
cases, vision may be substantially impaired. Other diseases, such as
Sjogren's disease and *cicatricial pemphigoid* manifest dry eye complications.

Although it appears that dry eye may result from a number of unrelated
25 pathogenic causes, all presentations of the complication share a common
effect, that is the breakdown of the pre-ocular tear film, which results in
dehydration of the exposed outer surface and many of the symptoms outlined
above (Lemp, *Report of the National Eye Institute/Industry Workshop on
Clinical Trials in Dry Eyes*, The CLAO Journal, volume 21, number 4, pages
30 221-231 (1995)).

Practitioners have taken several approaches to the treatment of dry eye. One common approach has been to supplement and stabilize the ocular tear film using so-called artificial tears instilled throughout the day. Other approaches include the use of ocular inserts that provide a tear substitute or stimulation of endogenous tear production.

Examples of the tear substitution approach include the use of buffered, isotonic saline solutions, aqueous solutions containing water soluble polymers that render the solutions more viscous and thus less easily shed by the eye. Tear reconstitution is also attempted by providing one or more components of the tear film such as phospholipids and oils. Phospholipid compositions have been shown to be useful in treating dry eye; see, e.g., McCulley and Shine, *Tear film structure and dry eye*, Contactologia, volume 20(4), pages 145-49 (1998); and Shine and McCulley, *Keratoconjunctivitis sicca associated with meibomian secretion polar lipid abnormality*, Archives of Ophthalmology, volume 116(7), pages 849-52 (1998). Examples of phospholipid compositions for the treatment of dry eye are disclosed in U.S. Patent Nos. 4,131,651 (Shah et al.), 4,370,325 (Packman), 4,409,205 (Shively), 4,744,980 and 4,883,658 (Holly), 4,914,088 (Glonek), 5,075,104 (Gressel et al.), 5,278,151 (Korb et al.), 5,294,607 (Glonek et al.), 5,371,108 (Korb et al.) and 5,578,586 (Glonek et al.). U.S. Patent No. 5,174,988 (Mautone et al.) discloses phospholipid drug delivery systems involving phospholipids, propellants and an active substance.

Another approach involves the provision of lubricating substances in lieu of artificial tears. For example, U.S. Patent No. 4,818,537 (Guo) discloses the use of a lubricating, liposome-based composition, and U.S. Patent No. 5,800,807 (Hu et al.) discloses compositions containing glycerin and propylene glycol for treating dry eye.

Although these approaches have met with some success, problems in the treatment of dry eye nevertheless remain. The use of tear substitutes, while temporarily effective, generally requires repeated application over the

course of a patient's waking hours. It is not uncommon for a patient to have to apply artificial tear solution ten to twenty times over the course of the day. Such an undertaking is not only cumbersome and time consuming, but is also potentially very expensive. Transient symptoms of dry eye associated with refractive surgery have been reported to last in some cases from six weeks to six months or more following surgery.

Aside from efforts directed primarily to the alleviation of symptoms associated with dry eye, methods and compositions directed to treatment of the dry eye condition have also been pursued. For example, U.S. Patent No. 5,041,434 (Lubkin) discloses the use of sex steroids, such as conjugated estrogens, to treat dry eye conditions in post-menopausal women; U.S. Patent No. 5,290,572 (MacKeen) discloses the use of finely divided calcium ion compositions to stimulate pre-ocular tear film production; and U.S. Patent No. 4,966,773 (Gressel et al.) discloses the use of microfine particles of one or more retinoids for ocular tissue normalization.

Some recent literature reports suggest that patients suffering from dry eye syndrome disproportionately exhibit the hallmarks of excessive inflammation in relevant ocular tissues, such as the lacrimal and meibomian glands. The use of various compounds to treat dry eye patients, such as steroids [e.g. U.S. Patent No. 5,958,912; Marsh, et al., *Topical nonpreserved methylprednisolone therapy for keratoconjunctivitis sicca in Sjogren syndrome*, Ophthalmology, 106(4): 811-816 (1999); Pflugfelder, et. al. U.S. Patent No. 6,153,607], cytokine release inhibitors (Yanni, J.M.; et. al. WO 0003705 A1), cyclosporine A [Tauber, J. *Adv. Exp. Med. Biol.* **1998**, 438 (Lacrimal Gland, Tear Film, and Dry Eye Syndromes 2), 969], and 15-HETE (Yanni et. al., US Patent No. 5,696,166), has been disclosed.

Summary of the Invention

The present invention is directed to methods for the treatment of dry eye and other disorders requiring the wetting of the eye, including symptoms of dry eye associated with refractive surgery such as LASIK surgery. According to the methods of the present invention, certain cytokine synthesis inhibitors are administered to a patient suffering from dry eye or other disorders requiring wetting of the eye. The cytokine synthesis inhibitors are preferably administered topically to the eye.

Brief Description of the Drawing

Fig. 1 shows the inhibition of hyperosmolarity-induced cytokine production from human corneal epithelial cells *in vitro* by SP-600125 and dexamethasone.

Detailed Description of the Invention

According to the present invention, inhibitors of cytokine synthesis by nonimmune resident ocular surface cells, including corneal and conjunctival epithelial and stromal cells, are administered to a patient suffering from dry eye. The compounds suitable for use in the present invention inhibit the synthesis of pro-inflammatory cytokines in nonimmune resident ocular surface cells by interfering with specific effectors of signaling cascades in these cells. Effectors of cytokine synthesis targeted for inhibition in the treatment of dry eye include mitogen-activated kinases (MAP kinase, p38 kinase), c-jun N-terminal kinase (JNK) and I-kappa kinase (IKK). Also, inhibitors of enzymes which convert precursors of the pro-inflammatory cytokines IL-1 β (ICE, IL-1 converting enzyme) and TNF α (TACE, TNF-alpha converting enzyme) to the active species or inhibit the translation of cytokine mRNA provide dry eye therapy. Cytokines promote further synthesis of pro-inflammatory cytokines

through activation of Janus family tyrosine kinase (JAK) and signal transducers and activators of transcription (STAT) and, therefore, inhibitors of JAKs and STATs provide treatment of dry eye. Inhibitors of activator protein-1 (AP-1) suppress cytokine synthesis in ocular surface cells and provide dry eye therapy. Additionally, ligands of retinoid X receptors (RXR) are known to suppress cytokine synthesis in epithelial cells and are suitable for use in the present invention.

The classes of cytokine synthesis inhibitors identified above are known. Inhibitors of MAP kinases (p38) include (5-(2-amino-4-pyrimidinyl)-4-(4-fluorophenyl)-1-(4-piperidinyl)imidazole) ["SB-220025"]. Inhibitors of JNK include anthra[1,9-cd]pyrazol-6(2H)-one ["SP-600125"]. Inhibitors of ICE include pralnacasan (HMR3480/VX-740). TNF mRNA translation inhibitors include (D)Arginyl-(D)Norleucyl-(D)Norleucyl-(D)Arginyl-(D)Norleucyl-(D)Norleucyl-(D)Norleucyl-Glycine-(D)Tyrosine-amide,acetate salt ["RDP58"]. NFkB inhibitors include 2-chloro-N-[3,5-di(trifluoromethyl)phenyl]-4-(trifluoromethyl)pyrimidine-5-carboxamide ["SP-100030"], and triflusal. AP-1 inhibitors include SP-100030. RXR agonists include bexarotene.

Preferred cytokine synthesis inhibitors for use in the present invention are JNK inhibitors and AP-1 inhibitors.

According to the methods of the present invention, a composition comprising one or more of the specified cytokine synthesis inhibitors and a pharmaceutically acceptable carrier for topical ophthalmic administration or implantation into the conjunctival sac or anterior chamber of the eye is administered to a mammal in need thereof. The compositions are formulated in accordance with methods known in the art for the particular route of administration desired.

The compositions administered according to the present invention comprise a pharmaceutically effective amount of one or more of the specified cytokine synthesis inhibitors. As used herein, a "pharmaceutically effective

amount" is one which is sufficient to reduce or eliminate signs or symptoms of dry eye or other disorders requiring the wetting of the eye. Generally, for compositions intended to be administered topically to the eye in the form of eye drops or eye ointments, the total amount of cytokine synthesis inhibitor
5 will be about 0.001 to 1.0% (w/w).

Preferably, the compositions administered according to the present invention will be formulated as solutions, suspensions and other dosage forms for topical administration. Aqueous solutions are generally preferred,
10 based on ease of formulation, as well as a patient's ability to easily administer such compositions by means of instilling one to two drops of the solutions in the affected eyes. However, the compositions may also be suspensions, viscous or semi-viscous gels, or other types of solid or semi-solid compositions. Suspensions may be preferred for cytokine synthesis inhibitors
15 which are sparingly soluble in water.

The compositions administered according to the present invention may also include various other ingredients, including but not limited to surfactants, tonicity agents, buffers, preservatives, co-solvents and viscosity building
20 agents.

Various tonicity agents may be employed to adjust the tonicity of the composition, preferably to that of natural tears for ophthalmic compositions. For example, sodium chloride, potassium chloride, magnesium chloride,
25 calcium chloride, dextrose and/or mannitol may be added to the composition to approximate physiological tonicity. Such an amount of tonicity agent will vary, depending on the particular agent to be added. In general, however, the compositions will have a tonicity agent in an amount sufficient to cause the final composition to have an ophthalmically acceptable osmolality (generally
30 about 150 – 450 mOsm, preferably 250 – 350 mOsm).

An appropriate buffer system (e.g., sodium phosphate, sodium acetate, sodium citrate, sodium borate or boric acid) may be added to the

compositions to prevent pH drift under storage conditions. The particular concentration will vary, depending on the agent employed. Preferably, however, the buffer will be chosen to maintain a target pH within the range of pH 6-7.5.

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Compositions formulated for the treatment of dry eye-type diseases and disorders may also comprise aqueous carriers designed to provide immediate, short-term relief of dry eye-type conditions. Such carriers can be formulated as a phospholipid carrier or an artificial tears carrier, or mixtures of both. As used herein, "phospholipid carrier" and "artificial tears carrier" refer to aqueous compositions which: (i) comprise one or more phospholipids (in the case of phospholipid carriers) or other compounds, which lubricate, "wet," approximate the consistency of endogenous tears, aid in natural tear build-up, or otherwise provide temporary relief of dry eye symptoms and conditions upon ocular administration; (ii) are safe; and (iii) provide the appropriate delivery vehicle for the topical administration of an effective amount of one or more of the specified cytokine inhibitors. Examples of artificial tears compositions useful as artificial tears carriers include, but are not limited to, commercial products, such as Tears Naturale®, Tears Naturale II®, Tears Naturale Free®, and Bion Tears® (Alcon Laboratories, Inc., Fort Worth, Texas). Examples of phospholipid carrier formulations include those disclosed in U.S. Patent Nos. 4,804,539 (Guo et al.), 4,883,658 (Holly), 4,914,088 (Glonek), 5,075,104 (Gressel et al.), 5,278,151 (Korb et al.), 5,294,607 (Glonek et al.), 5,371,108 (Korb et al.), 5,578,586 (Glonek et al.); the foregoing patents are incorporated herein by reference to the extent they disclose phospholipid compositions useful as phospholipid carriers of the present invention.

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Other compounds designed to lubricate, "wet," approximate the consistency of endogenous tears, aid in natural tear build-up, or otherwise provide temporary relief of dry eye symptoms and conditions upon ocular administration the eye are known in the art. Such compounds may enhance the viscosity of the composition, and include, but are not limited to: monomeric polyols, such as, glycerol, propylene glycol, ethylene glycol;

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polymeric polyols, such as, polyethylene glycol, hydroxypropylmethyl cellulose ("HPMC"), carboxy methylcellulose sodium, hydroxy propylcellulose ("HPC"), dextrans, such as, dextran 70; water soluble proteins, such as gelatin; and vinyl polymers, such as, polyvinyl alcohol, polyvinylpyrrolidone, povidone and carbomers, such as, carbomer 934P, carbomer 941, carbomer 940, carbomer 974P.

Other compounds may also be added to the ophthalmic compositions of the present invention to increase the viscosity of the carrier. Examples of viscosity enhancing agents include, but are not limited to: polysaccharides, such as hyaluronic acid and its salts, chondroitin sulfate and its salts, dextrans, various polymers of the cellulose family; vinyl polymers; and acrylic acid polymers. In general, the phospholipid carrier or artificial tears carrier compositions will exhibit a viscosity of 1 to 400 centipoises ("cps").

Topical ophthalmic products are typically packaged in multidose form. Preservatives are thus required to prevent microbial contamination during use. Suitable preservatives include: benzalkonium chloride, chlorobutanol, benzododecinium bromide, methyl paraben, propyl paraben, phenylethyl alcohol, edetate disodium, sorbic acid, polyquaternium-1, or other agents known to those skilled in the art. Such preservatives are typically employed at a level of from 0.001 to 1.0% w/v. Unit dose compositions of the present invention will be sterile, but typically unpreserved. Such compositions, therefore, generally will not contain preservatives.

The preferred compositions of the present invention are intended for administration to a human patient suffering from dry eye or symptoms of dry eye. Preferably, such compositions will be administered topically. In general, the doses used for the above described purposes will vary, but will be in an effective amount to eliminate or improve dry eye conditions. Generally, 1-2 drops of such compositions will be administered from once to many times per day.

A representative eye drop formulation is provided in Example 1 below.

Example 1

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Ingredient	Amount (% w/w)
Cytokine synthesis inhibitor	0.001-1.0
Polyoxyl 40 Stearate	0.1
Boric Acid	0.25
Sodium Chloride	0.75
Disodium Edetate	0.01
Polyquaternium-1	0.001
NaOH/HCl	q.s., pH = 7.4
Purified Water	q.s. 100%

The above composition is prepared by the following method. The batch quantities of boric acid, sodium chloride, disodium edetate, and polyquaternium-1 are weighed and dissolved by stirring in 90% of the batch quantity of purified water. The pH is adjusted to 7.4 ± 0.1 with NaOH and/or HCl. The batch quantity of the cytokine synthesis inhibitor as a stock solution is measured and added. Purified water is added to q.s. to 100%. The mixture is stirred for five minutes to homogenize and then filtered through a sterilizing filter membrane into a sterile recipient.

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Example 2: Inhibition of Hyperosmolarity-induced Cytokine Production from Human Corneal Epithelial Cells *in vitro*.

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The ocular tear film in dry eye is abnormally hyperosmolar, which is irritating to ocular surface cells. Treatment of human conjunctival epithelial cells with a hypertonic media elicits the production of pro-inflammatory

cytokines. A selective JNK inhibitor, anthra[1,9-cd]pyrazol-6(2H)-one, was evaluated for inhibition of hyperosmolarity-induced cytokine secretion from the transformed human corneal epithelial cell line, CEPI-17. CEPI-17 cells were grown in complete isotonic Keratinocyte Growth Medium (iso KGM) and plated in 48-well plates in iso KGM without hydrocortisone (iso KGM-HC). When the cells reached confluence, they were pre-treated for 1 hour with the compound at the indicated concentration in iso KGM-HC. The cells were then stimulated by hypertonic KGM-HC for 6 hours (additional 80mM NaCl added in iso KGM-HC) in the presence of the compound. Aliquots of the supernatants were assayed for IL-6, IL-8 by ELISA. Cytokine release was normalized by the amount of double stranded DNA (dsDNA) extracted from the cells. Percent inhibition of cytokine production was calculated by comparison with cytokine levels in vehicle-treated cells. The results are shown in Figure 1. Hyperosmolarity significantly induced IL-6 and IL-8 production from CEPI-17 cells under the conditions described. Dexamethasone significantly inhibited secretion of each cytokine at 100nM. Anthra[1,9-cd]pyrazol-6(2H)-one (SP-600125) dose-dependently inhibited both IL-6 ($IC_{50} = 12.6 \mu M$) and IL-8 ($IC_{50} = 3.7 \mu M$) production.

This invention has been described by reference to certain preferred embodiments; however, it should be understood that it may be embodied in other specific forms or variations thereof without departing from its special or essential characteristics. The embodiments described above are therefore considered to be illustrative in all respects and not restrictive, the scope of the invention being indicated by the appended claims rather than by the foregoing description.